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COMPOSITION FOR TREATMENT OF AND METHOD OF MONITORING HEPATITIS C VIRUS USING INTERFERON-TAU

This application claims priority to U.S. Provisional application Serial No. 60/219,128, and Japan Application No. 2000-317160 filed October 17, 2000, both of which are expressly incorporated by reference herein.

Field of the Invention

The present invention relates to the composition for treatment of conditions relating to hepatitis caused by hepatitis C virus (HCV) infection using Interferon-τ (IFN-τ). The present invention also relates to a method of monitoring treatment of HCV by measuring the blood levels of 2', 5'-oligoadenylate synthetase.

References

Ausubel, F.M., *et al.*, in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, Inc., Media, PA (1988).

Balzarini, J, *et al.*, *Biochem. Biophys. Res. Commun.* 178:563-569 (1991).

Bartol, F.F., *et al., Biol. Reprod.*33:745-759 (1985).

Bayne, M.L. *et al., Gene* 66:235-244 (1988).

Bazer, F.W., and Johnson, H.M., *Am. J. Reprod. Immunol.* 26:19-22 (1991).

Bazer, F.W., *et al.*, PCT publication WO/94/10313, published 11 May, 1994.

Beames, *et al., Biotechniques* 11:378 (1991).

Benvegnu, L., *et al., Cancer* 83:901-909 (1998).

Berenguer M., *et al., Adv. Gastroenterol. Hepatol. Clin. Nutr.* 1:2-21 (1996).

Charlier, M., *et al., Mol. Cell Endocrinol.* 76:161-171 (1991).

Choo, Q.–L., *et al., Science* 244, 359-362 (1989).

Choo, Q.–L., *et al., Proc. Natl. Acad. Sci. U.S.A.* 88, 2451-2455 (1991).

Clarke, B.E., *Baillieres Best Pract. Res. Clin. Gastroenterol.* 14:293-305 (2000).

Cotler, S.J., *et al., J. Viral Hepatitis* 7:211-217 (2000).

Cross, J.C., and Roberts, R.M., *Proc. Natl. Acad. Sci. USA* 88:3817-3821 (1991).

Di Bisceglie, A.M., *et al., Hepatology* 16:649-654 (1992).

Dieperink, E., *et al., Am. J. Psychiatry* 157:867-876 (2000).

Ecker, D.J., *et al., J. Biol. Chem.* 264:7715-7719 (1989).

Feher, Z., *et al., Curr. Genet.* 16:461 (1989).

Fernandez H., *et al., Eur. J. Epidemiol.* 2:1-14 (1986).

Godkin, J.D., *et al., J. Reprod. Fertil.* 65:141-150 (1982).

Gnatek, G.G., *et al, Biol. Reprod.* 41:655-664 (1989).

Hitzeman, R.A., *et al.,* U.S. Patent No. 4,775,622, issued October 4, 1988.

Helmer, S.D., *et al., J. Reprod. Fert.* 79:83-91 (1987).

Horiike N., *et al., C. Oncol. Rep.* 5:1171-1174 (1998).

Houglum, *Clin. Pharm.* 2:20-28 (1983).

Imakawa, K., *et al., Nature* 330:377-379 (1987).

Imakawa, K., *et al., Mol. Endocrinol.* 3:127 (1989).

Jarpe, M.A., *et al., Protein Engineering* 7:863-867 (1994).

Jimenez-Saenz, M., *et al., J. Gastroenterology and Hepatology* 15:567-569 (2000).

Klemann, S.W., *et al., Nuc. Acids Res.* 18:6724 (1990).

Koskinas J., *et al., J. Med. Virol.* 45:29-34 (1995).

Lechner, F., *et al., J. Exp. Med.* 191:1499-1512 (2000).

Ludwig, D.L., *et al., Gene* 132:33 (1993).

Magrin, S., *et al., Hepatology* 19, 273-279 (1994).

Maniatis, T., *et al.*, in MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1982).

Martal, J., *et al., J. Reprod. Fertil.* 56:63-73 (1979).

Martin, E.W., in DISPENSING OF MEDICATION: A PRACTICAL MANUAL ON THE FORMULATION AND DISPENSING OF PHARMACEUTICAL PRODUCTS (Mack Publishing Co., Easton, PA), 1976.

Mullis, K.B., U.S. Patent No. 4,683,202, issued 28 July 1987.

Mullis, K.B., *et al.*, U.S. Patent No. 4,683,195, issued 28 July 1987.

Oeda, K., *et al.*, U.S. Patent No. 4,766,068, issued August 23, 1988.

Ott, T.L., *et al., J. IFN Res.* 11:357-364 (1991).

Pawlotsky, J-M., *et al., J. Interferon and Cytokine Res.* 15:857-862 (1995).

Pearson, W.R. and Lipman, D.J., *PNAS* 85:2444-2448 (1988).

Pearson, W.R., *Methods in Enzymology* 183:63-98 (1990).

Reilly, P.R., *et al.*, BACULOVIRUS EXPRESSION VECTORS: A LABORATORY MANUAL, 1992.

Roberts, R.M., *et al., Endocrin. Rev.* 13:432-452 (1992).

Rutter, W.J., *et al.,* U.S. Patent No. 4,769, 238, issued September 6, 1988.

Saito, H., *et al., J. Viral Hepatitis* 7:64-74 (2000).

Sambrook, J., *et al.,* in MOLECULAR CLONING: A LABORATORY MANUAL, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989).

Shaw, K.J., *et al., DNA* 7:117 (1988).

Shen, L.P., *et al., Sci. Sin.* 29:856 (1986).

Shindo, M., *et al., Hepatology* 9:715-719 (1989)

Smith, P.K., *et al., Anal. Biochem.* 150:76 (1985).

Stewart, H.J., *et al, . Mol. Endocrinol.* 2:65 (1989).

Trepo, C., *J. Viral Hepatitis* 7:250-257 (2000).

Tyring, *et al.*, Interferon: Principles and Medical Applications, 1st Edition, Section VIII., pgs 399-408, 1992.

Vallet, J.L., *et al., Biol. Reprod.* 37:1307 (1987).

Whaley, A.E., *et al., J. Biol. Chem.* 269:10864-10868 (1994).

Wu, D.A., *et al., DNA* 10:201 (1991).

Background of the Invention

Hepatitis C virus (HCV) is a major public health problem affecting an estimated 170 million people worldwide and more than 10% of the population in some countries (Lechner, *et al.*, 2000). HCV is transmitted primarily by transfusion of infected blood and blood products (Cuthbert, *et al.*, 1994; Mansell, *et al.*, 1995). The Centers for Disease Control and Prevention estimate that HCV is responsible for 160,000 new cases of acute hepatitis in the United States each year. Therefore, an urgent medical need exists for an effective anti-HCV agent.

HCV is a positive-stranded, lipid-enveloped RNA virus of the Flaviviridae family, approximately ten thousand nucleotides in length (Choo, *et al.,* 1989). HCV, unlike hepatitis B virus, has no DNA intermediate, and therefore cannot be integrated into the host genome (Berenguer, *et al.*, 1996). Although HCV has been cloned, the virus has been difficult to culture *in vitro* (Trepo, 2000). HCV is extremely persistent, producing a chronic infection in 85% of infected individuals, although the mechanism of this persistence is unknown (Trepo, 2000).

Treatment of HCV is aimed at reducing inflammation and liver cell damage, thus preventing cirrhosis and hepatocellular carcinoma (Horiike, *et al.,* 1998; Benvegnu, *et al.,* 1998). Therapies that are currently available for HCV are only effective for a small subpopulation of infected patients (Magrin, *et al.,* 1994; Choo, *et al.*, 1991; Choo, *et al.*, 1989). IFN-α was introduced as therapy for chronic hepatitis C in the United States in 1991 and in Japan in 1992 (Saito, *et al.,* 2000). However, use of IFN-α in sufficient dosage to yield clinical efficacy (*i.e.*, at amounts of about $1x10^6$ units/treatment and above) is usually associated with a "flu-like" syndrome characterized by fever, headache, lethargy, arthalgias and myalgias (Tyring, *et al.*, 1992). At doses of $5\text{-}10x10^6$ units/treatment and above, other toxicities, such as nausea, vomiting, diarrhea and anorexia, become more frequent. Neuropsychiatric symptoms have also been reported in association with IFN-α treatment (Dieperink, *et al.*, 2000). In addition, some studies suggest that the efficacy of IFN-α treatment is not dose dependent (Saito, *et al.,* 2000), and that treatment with IFN-α is associated with the development or exacerbation of autoimmune disorders in patients with neoplasms or viral hepatitis (Jimenez-Saenz, *et al.*, 2000).

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a purine nucleoside analogue that has been found to interfere with viral mRNA synthesis and to inhibit *in vivo* and *in vitro* replication of a wide range of RNA and DNA viruses (Fernandez, *et al.*, 1986; Balzarini, *et al.*, 1991). Ribavirin has been shown to be efficient in normalizing aminotransferase levels, but has minor activity on serum HCV RNA titres in chronic hepatitis C patients (Di Bisceglie, *et al.*, 1992). Even the beneficial effects of ribavirin, however, are transient (Clarke, 2000; Koskinas, *et al.*, 1995), and because of severe side effects, ribavirin, in combination with IFN-α, can be difficult to tolerate (Cotler, *et al.,* 2000).

Because of the shortcomings associated with current HCV treatment methods, the inventors have set out to identify a new therapeutic candidate that will have more potent antiviral activity and less severe side effects.

Summary of the Invention

In one aspect, the invention includes an oral-delivery composition for use in treating HCV in a HCV-infected patient. The composition includes ovine Interferon-tau (OvIFN-τ), in a dosage effective to stimulate levels of 2', 5'-oligoadenylate synthetase (OAS) observed in the bloodstream 24 hours after administration of the composition. In one embodiment the composition also includes an oral-delivery vehicle containing IFN-τ and effective to release the IFN-τ in active form in the stomach. The composition provides a preferred dose of ovine IFN-τ between $10^8 - 10^{10}$ units.

The composition provides a preferred dose of ovine IFN-τ between $10^8 - 10^{10}$ units. In one embodiment, the dosage of ovine IFN-τ is greater than 1×10^8 Units/day. In another

embodiment, the dosage of ovine IFN-τ is greater than 2 x 10^8 Units/day. In yet another embodiment, the dosage of ovine IFN-τ is greater than 4 x 10^8 Units/day. In yet, still another embodiment, the dosage of ovine IFN-τ is greater than 1 x 10^9 Units/day. The dosage of ovine IFN-τ can be greater than 4 x 10^9 Units/day. Preferably, the dosage of ovine IFN-τ is greater than 7 x 10^9 Units/day.

In another aspect, the composition for treating HCV in a HCV-infected individual comprises ovine IFN-τ in a form that reaches the stomach, but not the tunica mucosa oris and at a dose effective to induce 2', 5'-oligoadenylate synthetase levels measured in the blood 24 hours after oral administration of the composition. A preferred dose is between about $10^8 - 10^{10}$ units.

In still another aspect, the composition of the invention includes ovine IFN-τ as an effective ingredient, where the composition avoids the absorption of ovine IFN-τ through the *tunica mucosa oris.*

In related aspects, a composition of the invention is for the treatment of hepatitis caused by HCV comprises ovine IFN-τ as an effective ingredient, and a 2', 5'-oligoadenylate synthetase activity inducer in animals other than sheep comprising ovine IFN-τ.

In still another aspect, the invention includes a method of monitoring treatment of HCV by oral administration of ovine IFN-τ. The method includes measuring the blood levels of 2', 5'-oligoadenylate synthetase prior to and after such oral administration, and if necessary, adjusting the dose of IFN-τ until a measurable increase in blood 2', 5'-oligoadenylate synthetase level, relative to the level observed prior to administration, is observed.

These and other objects and features of the invention will become more fully apparent when the following detailed description is read in conjunction with the accompanying drawings.

Brief Description of the Figures

Figure 1 shows OAS levels in mice whole blood following intraperitoneal (I.P.) or gastric administration (G.A.) of ovIFN-τ.

Figure 2 shows dose-dependent induction of blood OAS by gastric administration (G.A.) of ovIFN-τ.

Figures 3-5 illustrate HCV RNA and ALT levels in three human patients following oral administration of 4.9 x 10^8 units/day ovIFN-τ.

Figures 6 and 7 illustrate HCV RNA and ALT levels in two human patients following oral administration of 1.5 x 10^9 units/day ovIFN-τ.

Detailed Description of the Invention

I. Definitions

Hepatitis C virus or *HCV* refers to the viral species of which pathogenic types cause Non-A Non-B Hepatitis (NANBH), and attenuated types or defective interfering particles derived therefrom. The HCV genome is comprised of RNA. RNA containing viruses have relatively high rates of spontaneous mutation reportedly on the order of 10^{-3} to 10^{-4} per incorporated nucleotide. Since heterogeneity and fluidity of genotype are inherent in RNA viruses, there are multiple types/subtypes, within the HCV species which may be virulent or avirulent. The propagation, identification, detection, and isolation of various HCV types or isolates is documented in the literature.

Treating a condition refers to administering a therapeutic substance effective to reduce the symptoms of the condition and/or lessen the severity of the condition.

Oral refers to any route that involves administration by the mouth or direct administration into the stomach or intestines, including gastric administration.

OAS level refers to the concentration or activity of blood 2', 5'-oligoadenylate synthetase (OAS) protein.

Recombinant host cells, host cells, cells, cell lines, cell cultures, and other such terms denoting microorganisms or higher eukaryotic cell lines cultured as unicellular entities, are used interchangeably, and refer to cells which can be, or have been, used as recipients for recombinant vector or other transfer DNA, and include the progeny of the original cell transfected. It is understood that the progeny of a single parental cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to accidental or deliberate mutation. Progeny of the parental cell which are sufficiently similar to the parent to be characterized by the relevant property, such as the presence of a nucleotide sequence encoding a desired peptide, are included in the progeny intended yb this definition, and are covered by the above terms.

Operably linked refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence *operably linked* to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

An *open reading frame* is a region of a polynucleotide sequence which encodes for a polypeptide.

Ovine IFN-τ (ovIFN-τ) refers to a protein having the amino acid sequence as shown in Figure 4, and to proteins having amino acid substitutions and alterations such as neutral amino acid substitutions that do not significantly affect the activity of the protein. Preferably the sequence includes the ovine IFN-τ sequence of Figure 4 and the proteins with 90% sequence homology to the sequence shown in Figure 4.. Amino acid homology can be

determined using, for example, the ALIGN program with default parameters. This program is found in the FASTA version 1.7 suite of sequence comparison programs (Pearson and Lipman, 1988; Pearson, 1990; program available from William R. Pearson, Department of Biological Chemistry, Box 440, Jordan Hall, Charlottesville, VA).

II. Interferon-τ

The first IFN-τ to be identified was ovine IFN-τ (OvIFN-τ), as a 18-19 kDa protein. Several isoforms were identified in conceptus (the embryo and surrounding membranes) homogenates (Martal, *et al.*, 1979). Subsequently, a low molecular weight protein released into conceptus culture medium was purified and shown to be both heat labile and susceptible to proteases (Godkin, *et al.*, 1982). OvIFN-τ was originally called ovine trophoblast protein-one (oTP-1) because it was the primary secretory protein initially produced by trophectoderm of the sheep conceptus during the critical period of maternal recognition in sheep. Subsequent experiments have determined that OvIFN-τ is a pregnancy recognition hormone essential for establishment of the physiological response to pregnancy in ruminants, such as sheep and cows (Bazer and Johnson, 1991).

An IFN-τ cDNA obtained by probing a sheep blastocyst library with a synthetic oligonucleotide representing the N-terminal amino acid sequence (Imakawa, *et al.*, 1987) has a predicted amino acid sequence that is 45-55% homologous with IFN-αs from human, mouse, rat and pig and 70% homologous with bovine IFN-αII, now referred to as IFN-Ω. Several cDNA sequences have been reported which may represent different isoforms (Stewart, *et al.*, 1989; Klemann, *et al.*, 1990; and Charlier, M., *et al.*, 1991). All are approximately 1kb with a 585 base open reading frame that codes for a 23 amino acid leader sequence and a 172 amino acid mature protein. The predicted structure of IFN-τ as a four helical bundle with the amino and carboxyl-termini in apposition further supports its classification as a type I IFN (Jarpe, *et al.*, 1994).

Table 1

Overview of the Interferons

Aspects	Type I	Type I	Type I	Type II
Types	α & ω	β	τ	γ
Produced by:	leukocyte	fibroblast	trophoblast	lymphocyte
Antiviral	+	+	+	+
Antiproliferative	+	+	+	+
Pregnancy Signaling	-	-	+	-

While IFN-τ displays many of the activities classically associated with type I IFNs (see Table 1, above), considerable differences exist between it and the other type I IFNs. The most prominent difference is its role in pregnancy, detailed above. Also different is viral induction. All type I IFNs, except IFN-τ, are induced readily by virus and dsRNA (Roberts, *et al.*, 1992). Induced IFN-α and IFN-β expression is transient, lasting approximately a few hours. In contrast, IFN-τ synthesis, once induced, is maintained over a period of days (Godkin, *et al.*, 1982). On a per-cell basis, 300-fold more IFN-τ is produced than other type I IFNs (Cross and Roberts, 1991).

Other differences may exist in the regulatory regions of the IFN-τ gene. For example, transfection of the human trophoblast cell line JAR with the gene for bovine IFN-τ resulted in antiviral activity while transfection with the bovine IFN-Ω gene did not. This implies unique transacting factors involved in IFN-τ gene expression. Consistent with this is the observation that while the proximal promoter region (from 126 to the transcriptional start site) of IFN-τ is highly homologous to that of IFN-α and IFN-β; the region from –126 to –450 is not homologous and enhances only IFN-τ expression (Cross and Roberts, 1991). Thus, different regulatory factors appear to be involved in IFN-τ expression as compared with the other type I IFNs.

IFN-τ expression may also differ between species. For example, although IFN-τ expression is restricted to a particular stage (primarily days 13-21) of conceptus development in ruminants (Godkin, *et al.*, 1982), preliminary studies suggest that the human form of IFN-τ is constitutively expressed throughout pregnancy (Whaley, *et al.*, 1994).

A. Isolation of IFN-τ

OvIFN-τ protein may be isolated from conceptuses collected from pregnant sheep and cultured *in vitro* in a modified Minimum Essential Medium (MEM) as described by Godkin, *et al.*, (1982) and Vallet, *et al.*, (1987). The IFN-τ may be purified from the conceptus cultures by ion exchange chromotography and gel filtration. The homogeneity of isolated IFN-τ may be assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE; Maniatis, *et al.*, 1982; Ausubel, *et al.*, 1988), and determination of protein concentration in purified IFN-τ samples may be performed using the bicinchoninic (BCA) assay (Pierce Chemical Co., Rockford, IL; Smith, *et al.*, 1985).

B. Recombinant Production of IFN-τ

Recombinant IFN-τ protein may be produced from any selected IFN-τ polynucleotide fragment using a suitable expression system, such as bacterial or yeast cells. The isolation of IFN-τ nucleotide and polypeptide sequences is described in Bazer, *et al.* (1994). For example, Bazer, *et al.*, describe the identification and isolation of a human IFN-τ gene.

To make an IFN-τ expression vector, an IFN-τ coding sequence (*e.g,* SEQ ID NOS:1 or 3) is placed in an expression vector, *e.g.*, a bacterial expression vector, and expressed according to standard methods. Examples of suitable vectors include lambda gt11 (Promega, Madison WI); pGEX (Smith, *et al.*, 1985); pGEMEX (Promega); and pBS (Strategene, La Jolla CA) vectors. Other bacterial expression vectors containing suitable promoters, such as the T7 RNA polymerase promoter or the tac promoter, may also be used. Cloning of the OvIFN-τ synthetic polynucleotide into a modified pIN III omp-A expression vector is described in the Materials and Methods.

For the experiments described herein, the OvIFN-τ coding sequence present in SEQ ID NO:3 was cloned into a vector, suitable for transformation of yeast cells, containing the methanol-regulated alcohol oxidase (AOX) promoter and a Pho1 signal sequence. The vector was used to transform *P. pastoris* host cells and transformed cells were used to express the protein according to the manufacturer's instructions (Invitrogen, San Diego, CA).

Other yeast vectors suitable for expressing IFN-τ for use with methods of the present invention include 2 micron plasmid vectors (Ludwig, *et al.*, 1993), yeast integrating plasmids (YIps; *e.g.*, Shaw, *et al.*, 1988), YEP vectors (Shen, *et al.*, 1986), yeast centromere plasmids (YCps; *e.g.*), and other vectors with regulatable expression (Hitzeman, *et al.*, 1988; Rutter, *et al.*, 1988; Oeda, *et al.*, 1988). Preferably, the vectors include an expression cassette containing an effective yeast promoter, such as the MFα1 promoter (Bayne, *et al.*, 1988, GADPH promoter (glyceraldehyde-3-phosphate-dehydrogenase; Wu, *et al.*, 1991) or the galactose-inducible GAL10 promoter (Ludwig, *et al.*, 1993; Feher, *et al.*, 1989; Shen, *et al.*, 1986). The yeast transformation host is typically *Saccharomyces cerevisiae,* however, as illustrated above, other yeast suitable for transformation can be used as well (*e.g.*, *Schizosaccharomyces pombe, Pichia pastoris* and the like).

Further, a DNA encoding an IFN-τ polypeptide can be cloned into any number of commercially available vectors to generate expression of the polypeptide in the appropriate host system. These systems include the above described bacterial and yeast expression systems as well as the following: baculovirus expression (Reilly, *et al.*, 1992; Beames, *et al.*, 1991; Clontech, Palo Alto CA); plant cell expression, transgenic plant expression, and expression in mammalian cells (Clontech, Palo Alto CA; Gibco-BRL, Gaithersburg MD). The recombinant polypeptides can be expressed as fusion proteins or as native proteins. A number of features can be engineered into the expression vectors, such as leader

sequences which promote the secretion of the expressed sequences into culture medium. The recombinantly produced polypeptides are typically isolated from lysed cells or culture media. Purification can be carried out by methods known in the art including salt fractionation, ion exchange chromatography, and affinity chromatography. Immunoaffinity chromatography can be employed, as described above, using antibodies generated based on the IFN-τ polypeptides.

In addition to recombinant methods, IFN-τ proteins or polypeptides can be isolated from selected cells by affinity-based methods, such as by using appropriate antibodies. Further, IFN-τ peptides (*e.g.* SEQ ID NOS:2 or 4) may be chemically synthesized using methods known to those skilled in the art.

III. IFN-τ as a Treatment for HCV

Compositions and methods of the present invention may be used to therapeutically treat and thereby alleviate hepatitis caused by HCV. A person suffering from chronic hepatitis C infection may exhibit one or more of the following signs or symptoms: (a) elevated alanine aminotransferase (ALT), (b) positive test for anti-HCV antibodies, (c) presence of HCV as demonstrated by a positive test for HCV-RNA, (d) clinical stigmata of chronic liver disease, (e) hepatocellular damage, and/or (f) altered blood levels of 2', 5'-oligoadenylate synthetase. Such criteria may not only be used to diagnose hepatitis C, but can be used to evaluate a patient's response to drug treatment.

Interferon causes synthesis of the enzyme 2', 5'-oligoadenylate synthetase (OAS), which in turn, results in the degradation of viral mRNA (Houglum, 1983). OAS activates an RNase that cleaves cellular and viral RNAs, thereby inactivating viral replication (Kumar *et al.*, 1988). OAS is considered responsible, at least in part, for the antiviral state established in cells and plays a role in the elimination of HCV (Pawlotsky, *et al.*, 1995).

A. IFN Administered Orally and Intraperioneally Induce OAS

In experiments performed in support of the present invention and detailed in Examples 1 and 3, IFN-τ, administered orally, was tested for its ability to induce OAS. OvIFN-τ was administered either orally or intraperitoneally to mice or human patients. OAS activity in whole blood in mice was determined, and is shown in Figure 1, 24 hours after IFN-τ administration. Several human patients had 2 to 12 fold increases in their OAS enzyme activity levels as shown in Tables 3-6.

When OvIFN-τ was administered orally or intraperitoneally in mice, an increase in the OAS activity in whole blood was observed. When the effect of orally administered OvIFN-τ

and that of intraperitoneally administered OvIFN-τ in mice were compared, both administrations provided essentially the same whole blood OAS induction activity.

B. Orally Administered IFN-τ Induces OAS in a Dose-dependent Manner.

In experiments performed in support of the present invention and detailed in Example 2, IFN-τ, administered orally in mice, was tested for its ability to induce OAS in a dose-dependent manner. OvIFN-τ was orally administered in units of 0, $1x10^3$, $1x10^4$, $1x10^5$ to an upper part of a mouse stomach. Twelve hours after oral administration, whole blood was taken from a mouse heart and an OAS activity of whole blood was determined. As shown in Figure 2, the OAS activity in whole blood increased in a dose dependent manner.

Although it has already been established that IFN-τ is orally active (WO 96/28183), no exact determination has previously been made as to how IFN-τ was administered, or as to how IFN-τ is absorbed. In the present invention, IFN-τ was directly administered into the mouse stomach without any exposure to the *tunica mucosa oris*, conclusively esablishing that absorption through the stomach mucosal membrane effectively induces OAS activity. Direct absorption of IFN-τ from the stomach would diminish antibody formation against IFN-τ compared to IFN-τ absorbed through the oral mucosal membrane, particularly in the case of chronic administrations of IFN-τ.

In addition, the present invention describes the ability of ovine IFN-τ to increase 2', 5'-oligoadenylate synthase activity in mice and humans. Prior to this work, only mouse IFN-τ had been known to be effective in mice.

IV. Administration of IFN-τ

A. Pharmaceutical Compositions

Therapeutic preparations or medicaments containing IFN-τ or related polypeptides or proteins can be formulated and manufactured according to known methods for preparing pharmaceutically useful compositions (medicaments). Formulations comprising interferons or interferon-like compounds have been previously described (*e.g.*, Martin, 1976). In general, the IFN-τ-containing medicaments are formulated such that an effective amount of the IFN-τ is combined with a suitable carrier and/or excipient in order to facilitate effective administration of the composition. IFN-τ, or related polypeptides, may be administered to a patient in any pharmaceutically acceptable dosage form, including intravenous, intramuscular, intralesional, or subcutaneous injection. Specifically, compositions and methods used for other interferon compounds can be used for the delivery of these compounds.

In the case of compositions suitable for oral administration, tablets and capsules containing IFN-τ may be manufactured from IFN-τ (*e.g.*, lyophilized IFN-τ protein) and, optionally, additives such as pharmaceutically acceptable carriers (*e.g.*, lactose, corn starch, light silicic anhydride, microcrystalline cellulose, sucrose), binders (*e.g.*, alpha-form starch, methylcellulose, carboxymethylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, polyvinylpyrrolidone), disintegrating agents (*e.g.*, carboxymethylcellulose calcium, starch, low substituted hydroxy-propylcellulose), surfactants (*e.g.*, Tween 80, polyoxyethylene-polyoxypropylene copolymer), antioxidants (*e.g.*, L-cysteine, sodium sulfite, sodium ascorbate), lubricants (*e.g.*, magnesium stearate, talc), and the like.

Further, IFN-τ polypeptides can be mixed with a solid, pulverulent or other carrier, for example lactose, saccharose, sorbitol, mannitol, starch, such as potato starch, corn starch, millopectine, cellulose derivative or gelatine, and may also include lubricants, such as magnesium or calcium stearate, or polyethylene glycol waxes compressed to the formation of tablets. By using several layers of the carrier or diluent, tablets operating with slow release can be prepared.

Liquid preparations for oral administration can be made in the form of elixirs, syrups or suspensions, for example solutions containing from about 0.1% to about 30% by weight of IFN-τ, sugar and a mixture of ethanol, water, glycerol, propylene, glycol and possibly other additives of a conventional nature.

B. Dosage

An orally active IFN-τ pharmaceutical composition is administered in a therapeutically effective amount to an individual in need of treatment. The dose may vary considerably and is dependent on factors such as the seriousness of the disorder, the age and the weight of the patient, other medications that the patient may be taking and the like. This amount or dosage is typically determined by the attending physician. The dosage will typically be between about $1x10^5$ and $1x10^{10}$ units/day, preferably between about $1x10^8$ and $1.5x10^9$ units/day. It will be appreciated that because of its lower toxicity, IFN-τ can be administered at higher doses than, for example, IFN-α.

Disorders requiring a steady elevated level of IFN-τ in plasma will benefit from oral administration as often as about every two to four hours or administration via injection about every 12-24 hours, while other disorders may be effectively treated by administering a therapeutically-effective dose at less frequent intervals, *e.g.*, once every 48 hours. The rate of administration of individual doses is typically adjusted by an attending physician to enable

administration of the lowest total dosage while alleviating the severity of the disease being treated.

Once improvement of a patient's condition has occurred, a maintenance dose is administered if necessary. Subsequently, the dosage or the frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained.

C. Combination Therapies

It will, of course, be understood that the compositions and methods of this invention may be used in combination with other therapies. For example, the composition of ovIFN-τ for the treatment of HCV in a HCV-infected patient can be combined with an anti-viral agent such as ribavirin.

D. Monitoring

Treatment of HCV by oral administration of ovIFN-τ is monitored by measuring the blood levels of 2', 5'-oligoadenylate synthetase (OAS) prior to and following administration. The OAS levels can be monitored, for example, at 12, 24, and 48 hours after administration. If necessary, the dose of IFN-τ is adjusted until a measurable increase in blood OAS levels is observed, relative to the level observed prior to administration.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

The following examples illustrate, but are not intended in any way to limit the invention.

Materials and Methods

A. Production of OvIFN-τ

In one embodiment, a synthetic OvIFN-τ gene was generated using standard molecular methods (Ausubel, *et al.*, 1988) by ligating oligonucleotides containing contiguous portions of a DNA sequence encoding the OvIFN-τ amino acid sequence. The DNA sequence used may be either SEQ ID NO:1 or 3 or the sequence as shown in Imakawa, *et al.*, 1987. The resulting IFN-τ polynucleotide coding sequence may span position 16 through 531: a coding sequence of 172 amino acids.

In one embodiment, the full length synthetic gene *StuI/SStI* fragment (540 bp) may be cloned into a modified pIN III omp-A expression vector and transformed into a competent SB221 strain of *E. coli*. For expression of the IFN-τ protein, cells carrying the expression vector were grown in L-broth containing ampicillin to an OD (550 nm) of 0.1 – 1, induced with

IPTG (isopropyl-1-thio-b-D-galactoside) for 3 hours and harvested by centrifugation. Soluble recombinant IFN-τ may be liberated from the cells by sonication or osmotic fractionation.

For expression in yeast, the IFN-τ gene may amplified using polymerase chain reaction (PCR; Mullis, 1987; Mullis, *et al.*, 1987) with PCR primers containing *StuI* and *SacI* restriction sites at the 5' and 3' ends, respectively. The amplified fragments were digested with *StuI* and *SacII* and ligated into the *SacII* and *SmaI* sites of pBLUESCRIPT+(KS), generating pBSY-IFNτ. Plasmid pBSY-IFNτ was digested with *SacII* and *EcoRV* and the fragment containing the synthetic IFN-τ gene was isolated. The yeast expression vector pBS24Ub (Ecker, *et al.*, 1989) was digested with *SalI*. Blunt ends were generated using T4 DNA polymerase. The vector DNA was extracted with phenol and ethanol precipitated (Sambrook, *et al.*, 1989). The recovered plasmid was digested with *SacII*, purified by agarose gel electrophoresis, and ligated to the *SacII-EcoRV* fragment isolated from pBSY-IFN-τ. The resulting recombinant plasmid was designated pBS24Ub-IFNτ.

The recombinant plasmid pBS24Ub-IFNτ was transformed into *E. coli.* Recombinant clones containing the IFN-τ insert were isolated and identified by restriction enzyme analysis. IFN-τ coding sequences were isolated from pBS24Ub-IFNτ and cloned into a *Pichia pastoris* vector containing the alcohol oxidase (AOX1) promoter (Invitrogen, San Diego, CA). The vector was then used to transform *Pichia pastoris* GS115 His^- host cells and protein was expressed following the manufacturer's instructions. The protein was secreted into the medium and purified by successive DEAE-cellulose and hydroxyapatite chromatography to electrophoretic homogeneity as determined by SDS-PAGE and silver staining.

In one embodiment, the purified IFN-τ protein has a specific activity of about 0.29 to about 0.44×10^8 U/mg as measured by anti-viral activity on Madin-Darby bovine kidney (MDBK) cells. In another embodiment, the protein has a specific activity of about 4.9×10^8U/mg as measured by the anti-viral activity bioassay.

EXAMPLE 1

Induction of OAS with Orally and Intraperitoneally Administered Ovine IFN-τ to Mice

OvIFN-τ (4.99×10^8 units/mg protein; Pepgen Corp., California or Biological Process Development Facility, Dept. of Food Science and Technology, University of NE-Lincoln, Lincoln, NE; SEQ ID NO:4) was dissolved in 10% maltose solution to prepare ovIFN-τ Solution. The use of OvIFN-τ (SEQ ID NO:2) is also contemplated in the present invention. Two hundred microliters of ovIFN-τ solution was orally administered to ICR mice (average body weight approximately 30g, 6 weeks of age, female) using a 20 gauge disposable oral

sound (Fuchigami, Kyoto) to inject directly to an upper part of the stomach (gastric administration; GA).

For intraperitoneal administration (I.P.), 100 microliters of ovIFN-τ solution was used. Sample injection to an upper part of a stomach was confirmed by administration of a dye. Twenty-four hours after the administration, the mouse was anesthetized with Nembutal. Blood was taken from a heart of the mouse and an OAS activity in whole blood was determined by 2-5A RIA Kit (Eiken Chemical, Tokyo; Shindo *et al.*, 1989).

When the effect of orally administered 10^5 units of ovIFN-τ (τ GA) and that of intraperitoneally administered 10^5 units of OvIFN-τ (τ IP) were compared, both administrations provided essentially the same whole blood OAS induction activity. The results are shown in Figure 1.

EXAMPLE 2

Dose-dependent Induction of OAS by Oral Administration of IFN-τ in Mice

Using the same procedure as Example 1, OvIFN-τ was orally administered in units of 0, 10^3, 10^4, or 10^5 to an ICR mouse. Twelve hours after oral administration, whole blood was taken from a mouse heart and an OAS activity of whole blood was determined. As shown in Figure 2, the OAS activity in whole blood increased in a dose dependent manner.

EXAMPLE 3

Reduced ALT, Reduced HCV Viral Titer, and Induction of OAS by Oral Administration of IFN-τ in Human Patients

A. IFN-τ Preparation

On day one, one bottle of Ov-IFN-τ (SEQ ID NO:4) is removed from the refrigerator and the patient self-administers the proper volume of test material according to Table 2. Ov-IFN-τ (SEQ ID NO:2) may also be prepared and administered in the same manner.

Table 2

Recombinant Ov-IFN-τ Patient Dose Administration

Dose Group	Number of Patients	Ov-IFN-τ (mg/ml)	Volume (ml) per Dose (TID)	Total Daily Dose (ml)
I	6	1.0	0.33	1.0
II	6	1.0	1.0	3.0
III	6	1.0	3.0	9.0
IV	6	1.0	5.0	15.0

B. Patient Dosing Instructions

The patient keeps all vials of test material and syringes in the refrigerator maintained at 2 to 8 degrees centrigrade. Prior to the self-administration of medication, the patient removes one vial and one syringe from the refrigerator. The patient removes the cap from the tip of the syringe, places the tip of the syringe into the bottle of medication and withdraws the appropriate amount of drug into the syringe as instructed at the clinic on Day 1.

The patient places the tip of the syringe in the mouth and empties the contents of the syringe into the mouth by depressing the plunger. The patient then swallows the test material. The patient may then drink a glass of water. The patient notes on his/her diary card the date and time the dose of test material was administered.

The above steps are repeated three times per day at approximately eight-hour intervals: once in the morning, once at midday and once in the evening.

C. Results

The results of the human clinical trails in patients with HCV infections are shown in Tables 3-10 below, and graphically in Figures 3-7. An increase in OAS levels, and a decrease in both ALT and viral titer levels following oral ovine IFN-τ administration can be seen below.

Table 3

Human Clinical Trial Data – BB-IND9222 Dose Cohort I

PEPGEN OS NUMBER	Patient Initials/#	Timept.	Date Collected	HCV RT-PCR	ALT (IU/L)	2-5A (SERUM) pmol/dl 2-5A	2-5 (PBMC) pmol/5x10^6 PBMC/ml
180	PAB/001	*Screen*	11/17/2000	790,000	64	12.46	---
181	PAB/001	Day 1	12/1/2000	290,000	63	10.00	---
337	PAB/001	day 2/24hr.	12/2/2000	---	---	10.00	---
182	PAB/001	Day 3	12/3/2000	1,700,000	57	5.00	---
183	PAB/001	Day 8	12/8/2000	530,000	56	5.00	---
184	PAB/001	Day 15	12/15/2000	580,000	61	0.00	---
185	PAB/001	Day 22	12/22/2000	13,000	66	2.50	---
186	PAB/001	Day 29	12/29/2000	230,000	40	10.00	---
187	PAB/001	Day 43	1/12/2001	---	42	7.50	---
188	PAB/001	Day 57	1/26/2001	640,000	37	16.67	---
189	PAB/001	Day 71	2/9/2001	---	---	12/46	---
190	PAB/001	Day 85	2/23/2001	960,000	50	13.86	---
191	PAB/001	Day 113	3/23/2001	160,000	53	0.00	---
192	PAB/001	Day 169					---
193	MSM/002	*Screen*	11/27/2000	4,600,000	258	11.05	---
194	MSM/002	Day 1	12/11/2000	5,100,000	164	16.67	---

337	MSM/002	Day 2/24hr.	12/12/2000	---	---	10.00	---
195	MSM/002	Day 3	12/13/2000	6,300,000	154	29.30	---
196	MSM/002	Day 8	12/18/2000	5,100,000	133	33.08	---
197	MSM/002	Day 15	12/26/2000	9,100,000	100	54.62	---
198	MSM/002	Day 22	1/2/2001	---	103	51.54	---
199	MSM/002	Day 29	1/8/2001	8,600,000	91	28.60	---
200	MSM/002	Day 43	1/23/2001	---	86	12.46	---
201	MSM/002	Day 57	2/7/2001	3,400,000	82	18.77	---
202	MSM/002	Day 71	2/20/2001	---	---	36.15	---
203	MSM/002	Day 85	3/2/2001	3,700,000	49	26.14	---
204	MSM/002	Day 113	4/3/2001	3,800,000	64	42.31	---
205	MSM/002	Day 169					
206	DMA/003	*Screen*	12/1/2000	780,000	115	28.60	---
207	DMA/003	Day 1	12/12/2000	990,000	115	26.14	---
208	DMA/003	Day 3	12/14/2000	660,000	121	30.00	---
209	DMA/003	Day 8	12/19/2000	920,000	105	36.15	---
210	DMA/003	Day 15	12/26/2000	580,000	107	26.14	---
211	DMA/003	Day 22	1/2/2001	---	105	24.74	---
212	DMA/003	Day 29	1/9/2001	170,000	97	27.54	---
213	DMA/003	Day 43	1/22/2001	---	85	23.33	---
214	DMA/003	Day 57	2/5/2001	650,000	74	59.23	---
215	DMA/003	Day 71	2/20/2001	---	---	36.15	---
216	DMA/003	Day 85	3/5/2001	11,000	49	16.00	---
217	DMA/003	*Day 107*	3/27/2001	*880,000*	45	0.00	---
217	DMA/003	*Day 115*	4/4/2000	*50,000*	55	20.24	---
	DMA/003			*460,000*	47		--

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Table 4

HEPC CLINICAL TRIALS BB-IND9222 DOSE COHORT I

PEPGEN OS NUMBER	Patient Initials/#	Timept.	Date Collected	HCV RT-PCR	ALT (IU/L)	2-5A (SERUM) pmol/dl 2-5A	2-5 (PBMC) pmol/5x10^6 PBMC/ml
219	LER/004	*Screen*	12/12/2000	6,100,000	118	33.95	---
220	LER/004	Day 1	12/20/2000	6,000,000	108	33.95	---
221	LER/004	Day 3	12/22/2000	11,000,000	120	53.68	---
222	LER/004	Day 8	12/27/2000	1,900,000	109	29.51	---
223	LER/004	Day 15	1/3/2001	3,400,000	120	41.84	---
224	LER/004	Day 22	1/10/2001	---	94	34.74	---
225	LER/004	Day 29	1/17/2001	640,000	109	43.42	---
226	LER/004	Day 43	1/30/2001	---	99	49.74	---
227	LER/004	Day 57	2/13/2001	4,400,000	106	37.89	---
228	LER/004	Day 71	2/27/2001	---	---	81.00	---
229	LER/004	Day 85	3/14/2001	3,900,000	67	3.20	---
230	LER/004	Day 113		3,200,000	107		---
231	LER/004	Day 169					---
232	Z-I/005	*Screen*	12/20/2000	3,400,000	151	43.42	---
233	Z-I/005	Day 1	1/8/2001	4,600,000	134	43.42	---
338	Z-I/005	Day 2/24hr.	1/2/2001	---	144	45.00	---
234	Z-I/005	Day 3	1/10/2001	1,400,000	109	46.58	---
235	Z-I/005	Day 8	1/15/2001	4,000,000	94	12/93	---
236	Z-I/005	Day 15	1/22/2001	1,100,000	107	48.95	---
237	Z-I/005	Day 22	1/31/2001	---	107	47.37	---
238	Z-I/005	Day 29	2/7/2001	2,200,000	144	74.82	---
239	Z-I/005	Day 43	2/19/2001	---	111	26.10	---
240	Z-I/005	Day 57	3/5/2001	4,400,000	122	43.42	---
241	Z-I/005	Day 71	3/19/2001	---	---	10.00	---
242	Z-I/005	Day 85	4/4/2001	1,100,000	122	17.80	---
243	Z-I/005	Day 113		3,200,000	132		---
244	Z-I/005	Day 169					---
245	JRJ/006	*Screen*	1/5/2001	21,000,000	111	52.11	---
246	JRJ/006	Day 1	1/10/2001	8,500,000	104	21.90	---
247	JRJ/006	Day 3	1/12/2001	6,000,000	98	26.53	---
248	JRJ/006	Day 8	1/17/2001	950,000	124	24.21	---
249	JRJ/006	Day 15	1/24/2001	3,700,000	118	19.09	---
250	JRJ/006	Day 22	1/30/2001	---	109	22.07	---
251	JRJ/006	Day 29	2/7/2001	3,300,000	93	19.75	---
252	JRJ/006	Day 43	2/22/2001	---	122	24.88	---
253	JRJ/006	Day 57	3/7/2001	7,000,000	78	35.62	---
254	JRJ/006	Day 71	3/21/2001	---	---	52.92	---
255	JRJ/006	Day 85	4/4/2001	5,000,000	88	42.92	---
256	JRJ/006	Day 113		>5,000,000	109		---
257	JRJ/006	Day 169					---

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Table 5

HEPC CLINICAL TRIALS BB-IND9222 DOSE COHORT II

PEPGEN OS NUMBER	Patient Initials/#	Timept.	Date Collected	HCV RT-PCR	ALT (IU/L)	2-5A (SERUM) pmol/dl 2-5A	2-5 (PBMC) pmol/5x10^6 PBMC/ml
	AMC/007	*Screen*	2/2/2001	1,700,000	44	11.20	---
	AMC/007	Day 1	2/20/2001	1,300,000	48	18.40	---
	AMC/007	Day 3	2/22/2001	810,000	44	27.60	---
	AMC/007	Day 8	2/27/2001	630,000	50	42.40	---
	AMC/007	Day 15	3/6/2001	290,000	54	50.67	---
	AMC/007	Day 22	3/13/2001	---	53	94.50	---
	AMC/007	Day 29	3/20/2001	410,000	36	120.00	---
	AMC/007	Day 43	4/3/2001	---	29	81.33	---
	AMC/007	Day 57	4/17/2001	930,000	36	55.33	---
	AMC/007	Day 71	5/1/2001	---	---	51.33	---
	AMC/007	Day 85	5/15/2001				---
	AMC/007	Day 113	6/12/2001				---
	AMC/007	Day 169	8/7/2001				---
	ALW/008	*Screen*	2/2/2001	30,000,000	47	53.33	---
	ALW/008	Day 1	2/20/2001	3,000,000	38	10.00	---
	ALW/008	Day 3	2/22/2001	3,200,000	42	42.00	---
	ALW/008	Day 8	2/27/2001	5,400,000	31	14.40	---
	ALW/008	Day 15	3/6/2001	17,000,000	29	10.00	---
	ALW/008	Day 22	3/13/2001	---	27	10.40	---
	ALW/008	Day 29	3/20/2001	11,000,000	25	10.00	---
	ALW/008	Day 43	4/3/2001	---	40	14.40	---
	ALW/008	Day 57	4/17/2001	18,000,000	31	12.80	---
	ALW/008	Day 71	5/1/2001	---	---	16.40	---
	ALW/008	Day 85	5/15/2001				---
	ALW/008	Day 113	6/12/2001				---
	ALW/008	Day 169	8/7/2001				---
	DBF/012	*Screen*		5,300,000	84	28.80	---
	DBF/012	Day 1		9,300,000	77	26.00	---
	DBF/012	Day 3		9,400,000	71	10.00	---
	DBF/012	Day 8		7,900,000	86	53.33	---
	DBF/012	Day 15		9,100,000	67	108.00	---
	DBF/012	Day 22		---	64	42.67	---
	DBF/012	Day 29		9,900,000	58	52.00	---
	DBF/012	Day 43		---	61	58.00	---
	DBF/012	Day 57		15,000,000	70	61.33	---
	DBF/012	Day 71		---	---	168.00	---
	DBF/012	Day 85					---
	DBF/012	Day 113					---
	DBF/012	Day 169					---

Table 6

HEPC CLINICAL TRIALS BB-IND9222 DOSE COHORT II

PEPGEN OS NUMBER	Patient Initials/#	Timept.	Date Collected	HCV RT-PCR	ALT (IU/L)	2-5A (SERUM) pmol/dl 2-5A	2-5 (PBMC) pmol/5x10^6 PBMC/ml
	VCC/009	*Screen*	2/2/2001	5,100,000	113	17.20	---
	VCC/009	Day 1	2/21/2001	4,300,000	128	58.67	286.88
	VCC/009	Day 2/24hr.	2/22/2001	---	---	10.00	---
	VCC/009	Day 3	2/23/2001	3,500,000	126	18.40	218.57
	VCC/009	Day 8	2/28/2001	1,600,000	130	24.80	---
	VCC/009	Day 15	3/7/2001	2,200,000	118	25.20	624.38
	VCC/009	Day 22	3/14/2001	---	99	18.00	---
	VCC/009	Day 29	3/21/2001	1,500,000	93	30.67	1261.43
	VCC/009	Day 43	4/5/2001	---	72	15.20	---
	VCC/009	Day 57	4/18/2001	2,700,000	62	10.00	---
	VCC/009	Day 71	5/2/2001	---	---	18.40	---
	VCC/009	Day 85	5/16/2001			---	---
	VCC/009	Day 113	6/13/2001				---
	VCC/009	Day 169	8/8/2001				---
	HCM/010	*Screen*	2/2/2001	3,00,000	60	28.84	---
	HCM/010	Day 1	2/21/2001	5,000,000	47	12.31	998.1
	HCM/010	Day 2/24hr.	2/22/2001	---	---	---	---
	HCM/010	Day 3	2/23/2001	5,100,000	52	22.56	1336.67
	HCM/010	Day 8	2/28/2001	5,100,000	50	18.6	---
	HCM/010	Day 15	3/7/2001	5,300,000	49	30	1336.67
	HCM/010	Day 22	3/14/2001	---	49	47 08	---
	HCM/010	Day 29	3/21/2001	3,000,000	57	50	1524.76
	HCM/010	Day 43	4/4/2001	---	45	246	---
	HCM/010	Day 57	4/18/2001	4,300,000	59	16.67	---
	HCM/010	Day 71	5/2/2001	---	---	15.26	---
	HCM/010	Day 85	5/16/2001				---
	HCM/010	Day 113	6/13/2001				---
	HCM/010	Day 169	8/8/2001				
	CLR/011	*Screen*	2/5/2001	12,000,000	58	10.00	---
	CLR/011	Day 1	2/21/2001	19,000,000	66	30.00	960.48
	CLR/011	Day 3	2/23/2001	28,000,000	55	11.05	922.86
	CLR/011	Day 8	2/28/2001	>5,000,000	55	12.46	---
	CLR/011	Day 15	3/7/2001	23,000,000	63	12.46	1035.71
	CLR/011	Day 22	3/14/2001	---	65	19.82	---
	CLR/011	Day 29	3/21/2001	13,000,000	58	10.00	998.1
	CLR/011	Day 43	4/4/2001	---	63	36.00	---
	CLR/011	Day 57	4/18/2001	18,000,000	61	20.80	---
	CLR/011	Day 71	5/2/2001	---	---	10.00	---
	CLR/011	Day 85	5/16/2001				---
	CLR/011	Day 113	6/13/2001				---
	CLR/011	Day 169	8/8/2001				---

Table 7

Dose Group 1 (0.33 mg TID) – 24 Hour Serum Collection PCR Assays (HCV RNA)

Patient ID	Screen	Day 1	Day 3	Day 8	Day 15	Day 22	Day 29	Day 57	Day 85	Day 113	Day 169
001 PAB	790,000	290,000	1,700,000	530,000	580,000	13,000[1]	230,000	640,000	960,000	160,000	110,000
002 MSM	4,600,000	5,100,000	6,300,000	5,100,000	9,100,000		8,600,000	3,400,000	3,700,000	3,800,000	1,900,000
003 DMA	780,000	990,000	660,000	920,000	580,000		170,000	650,000	11,000	880,000[2] 50,000[3] 460,000[4]	340,000[5]
004 LER	6,100,000	6,000,000	11,000,000	1,900,000	3,400,000		640,000	4,400,000	3,900,000	3,200,000	3,800,000
005 Z-I	3,400,000	4,600,000	1,400,000	4,000,000	1,100,000		2,200,000	4,400,000	1,100,000	3,200,000	1,300,000
006 JRJ	21,000,000	8,500,000	6,000,000	950,000	3,700,000		3,300,000	7,000,000	5,000,000	5,100,000	>5,000,000

[1] PCR Assay not scheduled for Day 22
[2] Day 1 of Retreat
[3] Day 8 of Retreat
[4] Day 29 of Retreat
[5] Day 164 of Retreat

Table 8

ALT Values (IU/L) – Dose Group 1

Patient ID	Screen	Day 1	Day 3	Day 8	Day 15	Day 22	Day 29	Day 43	Day 57	Day 85	Day 113	Day 169
001 PAB	64	63	57	56	61	66	40*	42*	37*	50	53	50
002 MSM	258	164	154	133	100	103	91	86	82	49	64	61
003 DMA	115	115	121	105	107	105	97	85	74	49	45	51
004 LER	118	108	120	109	120	94	109	99	106	67	107	120
005 Z-I	151	134	144	109	94	107	107	144	111	122	132	
006 JRJ	111	104	98	124	118	109	93	122	78	88	109	
Mean	116.71	98.43	99.57	92	87.86	86.57	82.83	89.33	75.17	72.86	89	64.43

Std												

[1]Normal ALT (range = 1 – 45)
[2]Day 1 of Retreat.
[3]Day 8 of Retreat.
[4]Day 29 of Retreat.
[5]Day 164 of Retreat
[6]Day 192 of Retreat

Table 9

Dose Group 2 (1.0 mg TID) – 24 Hour Serum Collection PCR Assays (HCV RNA)

Patient ID	Screen	Day 1	Day 3	Day 8	Day 15	Day 29	Day 57	Day 85	Day 113	Day 169
007 AMC	1,700,000	1,300,000	810,000	630,000	290,000	410,000	930,000	900,000	310,000	
008 ALW	30,000,000	3,000,000	3,200,000	5,400,000	17,000,000	11,000,000	18,000,000	7,700,000	11,000,000	
009 VCC	5,100,000	4,300,000	3,500,000	1,600,000	2,200,000	1,500,000	2,700,000	1,700,000	670,000	
010 HMC	3,000,000	5,000,000	5,100,000	5,100,000	5,300,000	3,000,000	4,300,000	3,100,000	4,400,000	
011 CLR	12,000,000	19,000,000	28,000,000	>5,000,000	23,000,000	13,000,000	18,000,000	9,400,000	8,200,000	
012 DBF	5,300,000	9,300,000	9,400,000	7,900,000	9,100,000	9,900,000	15,000,000	9,500,000	16,000,000	

Table 10

ALT Values (IU/L) – Dose Group 2

Patient ID	Screen	Day 1	Day 3	Day 8	Day 15	Day 22	Day 29	Day 43	Day 57	Day 85	Day 113	Day 169
007 AMC	44**	48	44	50	54	53	36*	29*	36*	37*	49	
008 ALW	47	38*	42*	31*	29*	27*	25*	40*	31*	31*	25*	
009 VCC	113	128	126	130	118	99	93	72	62	38*	34*	
010 HMC	60	47	52	50	49	49	57	45	59	51	58	
011 CLR	58	66	55	55	63	65	58	63	61	60	61	
012 DBF	84	77	71	86	67	64	58	61	70	89	92	

*Normal ALT Value (range = 1 – 45)
**Normal ALT Value for female 67 years of age (4 – 40)